

WHAT IS CLAIMED IS:

1 1. A method for mapping a site of post-translational modification on a
2 post-translationally modified polypeptide, said method comprising:

3 (a) site-specifically cleaving a peptide bond of the post-translationally
4 modified polypeptide with an endopeptidase at said site of post-translational modification to
5 produce a degraded post-translationally modified polypeptide; and

6 (b) after step (a), determining said site of post-translational modification.

1 2. The method of claim 1, wherein said post-translational modification is
2 selected from phosphorylation, sulfonation, glycosylation, acetylation, methylations, ADP-
3 ribosylation, methionine oxidation, cysteine oxidation, and cysteine lipidation.

1 3. The method of claim 1, wherein said post-translational modification is
2 phosphorylation of an amino acid selected from tyrosine, serine, and threonine.

1 4. The method of claim 1, wherein said post-translational modification is
2 sulfonation of a tyrosine.

1 5. The method of claim 1, wherein said site of post-translational
2 modification is determined by a method comprising determining the mass spectrometry
3 fragmentation pattern of the degraded post-translationally modified polypeptide.

1 6. The method of claim 1, wherein said endopeptidase is a serine protease
2 comprising an active site that specifically binds to said post-translational modification.

1 7. The method of claim 6, wherein said serine protease is subtilisin.

1 8. A serine protease which site-specifically cleaves a peptide bond of a
2 post-translationally modified polypeptide at a site of post-translational modification, wherein
3 said serine protease comprises an active site that binds to said site of post-translational
4 modification.

1 9. The serine protease of claim 8, wherein said post-translational
2 modification is selected from phosphorylation, sulfonation, glycosylation, and acetylation.

1 10. The serine protease of claim 8, wherein said post-translational
2 modification is phosphorylation of an amino acid selected from tyrosine, serine, and
3 threonine.

1 11. The serine protease of claim 8, wherein said post-translational
2 modification is sulfonation of a tyrosine.

1 12. The serine protease of claim 8, wherein said serine protease is
2 subtilisin.

1 13. The serine protease of claim 8, wherein said serine protease is encoded
2 by a nucleic acid sequence that hybridizes under highly stringent hybridization conditions to
3 a nucleic acid encoding a polypeptide comprising an amino acid sequence of Figure 1,
4 wherein the hybridization reaction is incubated at 42°C in a solution comprising 50%
5 formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x SSC and
6 0.1% SDS.

1 14. The serine protease of claim 8, wherein said serine protease comprises
2 a subsequence having at least 70% amino acid sequence identity to an amino acid sequence
3 of Figure 1.

1 15. The serine protease of claim 8, wherein said serine protease comprises
2 a subsequence having at least 70% amino acid sequence identity to an amino acid sequence
3 of Figure 1 and contains at least one amino acid substitution selected from P129G, E156R,
4 S191K, G166K, and G127S.

1 16. The serine protease of claim 8, wherein said serine protease is encoded
2 by an expression vector.

1 17. A host cell comprising the expression vector of claim 16.

1 18. An endopeptidase that site-specifically cleaves a peptide bond of a
2 post-translationally modified polypeptide at a site of post-translational modification, said
3 endopeptidase produced by a method comprising:

4 (a) introducing one or more point mutations to a model endopeptidase at one
5 or more candidate amino acid positions in an active site of said model endopeptidase to

6 produce a plurality of candidate endopeptidases, wherein at least one of said plurality of
7 candidate endopeptidases is an endopeptidase that site-specifically cleaves a peptide bond of
8 a post-translationally modified polypeptide at a site of post-translational modification; and
9 (b) identifying said endopeptidase that site-specifically said peptide bond of
10 said post-translationally modified polypeptide cleaves at said site of post-translational
11 modification.

1 19. The endopeptidase of claim 18, wherein said model endopeptidase
2 comprises a subsequence having at least 70% amino acid sequence identity to an amino acid
3 sequence of Figure 1.

1 20. The endopeptidase of claim 18, wherein said model endopeptidase is
2 encoded by a nucleic acid sequence that hybridizes under highly stringent hybridization
3 conditions to a nucleic acid encoding a polypeptide comprising an amino acid sequence of
4 Figure 1, wherein the hybridization reaction is incubated at 42°C in a solution comprising
5 50% formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x
6 SSC and 0.1% SDS.

1 21. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is selected from P129, E156, S191, G166, and G127.

1 22. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is P129 and said point mutation is a glycine or alanine substitution.

1 23. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is E156 and said point mutation is an arginine substitution.

1 24. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is E156 and said point mutation is a lysine substitution.

1 25. The endopeptidase of claim 18, wherein said one or more candidate
2 amino acid positions is P129 and E156, wherein said point mutation is glycine at p129 and
3 arginine at E156.

1 26. The endopeptidase of claim 18, wherein, before step (a), said one or
2 more candidate amino acid positions are identified by a method comprising:

(i) generating a three-dimensional structure of said model endopeptidase active site;
(ii) generating a three-dimensional structure of said post-translationally modified polypeptide;
(iv) comparing the three-dimensional structure of said model endopeptidase active site with said post-translationally modified polypeptide, thereby identifying one or more candidate amino acid positions that, upon introduction of one or more point mutations at one or more of said candidate amino acid positions, produces a plurality of candidate endopeptidases, wherein at least one of said plurality of candidate endopeptidases is said endopeptidase that site-specifically said peptide bond of said post-translationally modified polypeptide cleaves at said site of post-translational modification.

27. An isolated nucleic acid encoding a endopeptidase which site-specifically cleaves a peptide bond of a post-translationally modified polypeptide at a site of post-translational modification and which comprises one or more point mutations at one or more amino acid positions within the endopeptidase active site,

wherein said isolated nucleic acid hybridizes under highly stringent hybridization conditions to a nucleic acid sequence of Figure 2, wherein the hybridization reaction is incubated at 42°C in a solution comprising 50% formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x SSC and 0.1% SDS.

28. An expression vector comprising the nucleic acid of claim 27.

29. A host cell transfected with the vector of claim 28.

30. An isolated nucleic acid encoding a endopeptidase which site-specifically cleaves a polypeptide backbone amide bond of a post-translationally modified polypeptide at a site of post-translational modification and which comprises one or more point mutations at one or more amino acid positions within the endopeptidase active site, wherein said isolated nucleic acid comprises a subsequence having at least 70% nucleic acid sequence identity to a nucleic acid sequence of Figure 2.

31. An expression vector comprising the nucleic acid of claim 30.

32. A host cell transfected with the vector of claim 30.